

REMARKS

This Preliminary Amendment is submitted to place the application in better condition for examination and to facilitate the prosecution thereof. General support for new claims 108-148 can be found throughout the specification. Support for claim 108 can be found at page 5, line 12, through page 6, line 14. Support for claim 108 can be found at page 22, lines 7-10. Support for claim 110 can be found at page 32, lines 21-33. Support for claims 111-113 can be found at page 24, lines 2-5. Support for claims 114-115 can be found at page 23, lines 22-29. Support for claim 116 can be found at page 22, lines 19-22. Support for claim 117 can be found at page 24, lines 2-5. Support for claim 118 can be found at page 21, line 24 through page 22, line 13. Support for claim 119 can be found at page 19, lines 16-23. Support for claim 120 can be found at page 31, lines 14-16. Support for claim 121 can be found at page 19, lines 29-32. Support for claims 122-123 can be found at page 20, lines 13-16. Support for claim 124 can be found at page 26, line 11. Support for claims 125-126 can be found at page 23, lines 1-6. Support for claim 127 can be found at page 22, lines 4-6. Support for claims 128-130 can be found at page 76, line 25 through page 78, line 15. Support for claim 131 can be found at page 5, line 12 through page 6, line 15. Support for claims 132-134 can be found at page 24, lines 2-5. Support for claims 135-138 can be found at page 22, lines 19-22. Support for claim 139 can be found at page 24, lines 2-5 of the specification. Support for claim 140 can be found at page 14, lines 23-28. Support for claim 141 can be found at page 58, lines 31-33. Support for claim 142 can be found page 6, line 29 through page 7, line 20. Support for claim 143 can be found at page 26, line 11. Support for claims 144-145 can be found at page 9, line 23 through page 10, line 31. Support for claim 146 can be found at page 11, lines 2-8. Support for claim 147 can be found at page 11, line 11 through page 12, line 24. Support for claim 148 can be found at page 12, lines 26-30. No new matter has been added.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant : Marc Vidal et al
Serial No. :
Filed :
Page : 12

Attorney's Docket

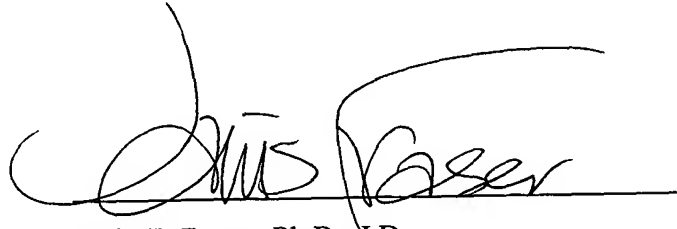
0974-239005 / MGH-0792.3
Vidal

Applicant asks that all claims be examined. Please apply any charges or credits to
Deposit Account No. 06-1050, referencing attorney docket no. 10974-239005.

Respectfully submitted,

Date:

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Version with markings to show changes made

In the specification:

Add the following at page 1, between line 3 and line 4,:

-- RELATED APPLICATIONS

This application is a continuation of U.S. Serial No. 08/923,274, filed September 4, 1997, now allowed; which is a continuation of U.S. Serial No. 08/420,525, filed April 11, 1995, now abandoned.

The paragraph beginning at page 33, line 28 has been amended as follows:

Fig. 2 is a map of the plasmid p2.5. A portion of pPC97 (left panel) containing a polylinker, is represented by SEQ ID NO: 7. The amino acid sequence encoded by this portion of pPC97 is represented by SEQ ID NO: 8. A portion of pPC86 (right panel), containing a polylinker, is represented by SEQ ID NO: 9. The amino acid sequence encoded by this portion of pPC86 is represented by SEQ ID NO: 10.

The paragraph beginning at page 35, line 27 has been amended as follows:

Fig. 10A is a schematic representation of plasmids into which the CYH2 counterselectable marker was inserted. A portion of pPC97 (left panel), containing a polylinker, is represented by SEQ ID NO: 7. The amino acid sequence encoded by this portion of pPC97 is represented by SEQ ID NO: 8. A portion of pPC86 (right panel), containing a polylinker, is represented by SEQ ID NO: 9. The amino acid sequence encoded by this portion of pPC86 is represented by SEQ ID NO: 10.

The paragraph beginning at page 37, line 31 has been amended as follows:

Fig. 21 is a schematic representation of the Marked Box 2 domain and the mutations obtained with the two-step selection method. The amino acid sequences of the Marked Box 2 domains of E2F5, E2F4, E2F2, and E2F1 are represented by SEQ ID NOS: 11-15, respectively.

The amino acid sequences of the Marked Box 2 domains of the alleles E2F1-20, E2F1-30, E2F1-32, and E2F1-65 are represented by SEQ ID NOS: 16-19, respectively.

The paragraph beginning at page 44, line 8 has been amended as follows:

Construction of Plasmids for Producing Hybrid Proteins: Plasmids p97.CYH2 and pMV257 are useful in the invention for producing hybrid proteins having a GAL4-DB or AD, respectively, fused to a potential interacting molecule of interest (Fig. 10B). These plasmids are produced by inserting a sequence encoding CYH2 into pPC97 (for DB plasmids) or [pPC97] pPC86 (for AD plasmids) (Fig. 10A). Both p97.CYH2 and pMV257 have (i) a yeast *ARS4* origin of replication; (ii) a yeast *CEN6* centromeric sequence; (iii) a selectable marker (e.g., *LEU2* for pPC97, and *TRP1* for pPC86); (iv) a yeast *ADHI* promoter and terminator; (v) a GAL4-DB (for pPC97) or a GAL4-AD (for pPC86); (vi) an SV40 large T antigen sequence encoding a nucleolar signal sequence positioned in frame with the DB or AD domain; (vii) a bacterial origin of replication; and (viii) a *CYH2* counterselectable marker. Those skilled in the art recognize that numerous similar plasmids can be used to produce hybrid proteins. For example, hybrid proteins that include the DB or AD of VP16 (from Herpes Simplex Virus or Ace1 can be produced with plasmids having, in place of the GAL4-DB or -AD, sequences encoding the VP16 or Ace1 DB or Ace1 AD. Similarly selectable markers other than *Leu2* and *Trp1* can be used. These plasmids can be constructed with conventional molecular biology methods. Generally, in order to select for a yeast cell containing one of these plasmids, the yeast cell should not, in the absence of the plasmid, express a functional gene product which corresponds to the selectable marker. For example, a yeast cell into which p97.CYH2 is transformed should have a *leu2* mutation; thus, a transformant containing p97.CYH2 can be selected on a medium which lacks leucine. The yeast strains MaV103 and MaV203 are particularly useful in conjunction with p97.CYH2 and pMV257.

In the claims:

Claims 2-107 have been cancelled.